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(57) Abstract

The present invention is a method of treating hepatitis in which granulocyte/macrophage-colony stimulating factor (GM-CSF) is administered to a patient in need of such treatment with a therapeutically effective amount alone or in conjunction with α-Interferon (INF-α).

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TREATMENT OF HEPATITIS WITH GM-CSF

FIELD OF THE INVENTION

The present invention relates to the treatment of hepatitis by administering therapeutically effective amounts of granulocyte-macrophage (GM-CSF), particularly human GM-CSF.

BACKGROUND OF THE INVENTION

Hepatitis is an illness involving inflammation of the liver which can be characterized as acute or chronic depending upon the length and severity of the illness. Four types of hepatitis viral agents have been implicated, namely, hepatitis A virus (HAV), hepatitis B virus (HBV), hepatitis C virus (HCV), and the HBV-associated delta agent (HDV). Although these agents can be distinguished by their antigenic properties, all four types produce clinically similar illnesses. These range from asymptomatic and unapparent to fulminant and fatal acute infections common to all five types, on the one hand, and from subclinical persistent infections to rapidly progressive chronic liver disease with cirrhosis and even hepatocellular carcinoma, common to the bloodborne types HBV, HCV and HDV.

Early indicators of infection by a hepatitis viral agent is an increase in serum aminotransferases aspartate aminotransferase (AST) and alanine aminotransferase (ALT) which precedes a rise in bilirubin level. Peak levels of each vary from 400 to 4000 IU or more. These levels are usually reached at the time the patient is clinically icteric and diminish progressively during the recovery phase of acute hepatitis.

HAV is an RNA virus. Antibodies to HAV (anti-HAV) can be detected during acute illness when serum ALT and AST activity is elevated and fecal HAV shedding is still occurring. This early antibody response is predominantly of the IgM class and persists for several months. During convalescence, however, anti-HAV of the IgG class

becomes the predominant antibody. Therefore, the diagnosis of hepatitis A is made during acute illness by demonstrating high-titer anti-HAV of the IgM class. Following acute illness, anti-HAV of the IgG class remains detectable indefinitely, and patients with serum anti-HAV are immune to reinfection.

HBV is a DNA virus which belongs to the hepadnavirus family. HBV can be detected by a number of methods, such as detection of the hepatitis B surface antigen (HBsAg), the detection of the antigen expressed on the surface of the nucleocapsid, referred to as hepatitis B core antigen (HBcAg), and the corresponding antibody antiHBc. HBcAg does not cross-react with HBsAg. A third antigen associated with hepatitis B is hepatitis B e antigen (HBeAg). In every individual with acute hepatitis B infection, HBeAg develops transiently, early in the course of illness, but persistent HBeAg positivity correlates with ongoing viral replication and may be associated with continuing disease activity in chronic hepatitis; its disappearance may be a harbinger of biochemical improvement and resolution of infection. Like HBeAg, serum HBV DNA and DNA polymerase are indicators of HBV replication but they are more sensitive. In self-limited HBV infections, HBeAg becomes undetectable shortly after peak elevations in ALT and AST activity, before the disappearance of HBsAg, and antiHBe then becomes detectable, coinciding with a period of relatively lower infectivity. In protracted HBV infection, HBeAg may remain detectable, indicating persistent replicative infection. When HBeAg is absent and anti-HBe is present in chronic hepatitis B, infection is usually nonreplicative. Occasionally, nonreplicative HBV infection converts back to replicative infection. Such spontaneous reactivations are accompanied by reexpression of HBeAg and HBV DNA as well as by exacerbations of liver injury.

Chronic hepatitis refers to any of several types of hepatitis persisting for more than six months, often progressing to cirrhosis of the liver and may be designated chronic hepatitis A, B, or C depending upon their origin. Examples of chronic hepatitis are three related disorders, namely, chronic persistent hepatitis, chronic lobular hepatitis,

and chronic active hepatitis. These are characterized by a combination of hepatocyte necrosis and inflammation of varying severity persisting for more than 6 months.

The clinically most important disorder, chronic active hepatitis, may lead to hepatic failure and death or result in the development of cirrhosis. Chronic active hepatitis is a major late complication of acute hepatitis B occurring is a small proportion of acute cases but more common in those with chronic infection. Certain clinical and laboratory features suggest progression of acute hepatitis to chronic active hepatitis: (1) lack of complete resolution of clinical symptoms of anorexia, weight loss, fatigue and the persistence of hepatomegaly; (2) the presence of bridging or multilobular hepatic necrosis on liver biopsy during protracted, severe acute viral hepatitis; (3) failure of the serum ALT and AST, bilirubin, and globulin levels to return to normal within 6 to 12 months following the acute illness; and (4) the continued presence of HBsAg and/or HBV-DNA and DNA polymerase 6 months or more after acute hepatitis, suggesting chronic viral infection of the liver.

Most human subjects with active liver disease will also be positive for HBeAg. This serological marker is invariably present at the onset of the chronic HBsAg carrier state. With time, HBeAg may disappear and replaced by anti-HBe. This seroconversion is usually, but no always, accompanied by a sustained decrease in the activity of the chronic liver disease. Those subjects with anti-HBe who have ongoing chronic liver disease typically have other serological evidence of active viral replication, either low and intermittent levels of HBV-DNA and DNA polymerase or hepatitis B core antigen inliver. Another serological marker for the presence of active HBV replication and accompanying chronic hepatitis disease activity is IgM anti-HBc. This antibody may be present when HBV-DNA, DNA polymerase and HBeAg are no longer detectable or are present in low titer.

HCV is a single-stranded RNA virus with a genome of approxiamately 10,000 nucleotides. It has no homology with HBV, or retroviruses, or other hepatitis viruses however, its genome, size, and stability are consistent with its inclusion in the togavirus family of lipid-

enveloped agents that includes the arboviruses (such as yellow fever and dengue viruses) and rubella viruses. A diagnostic test for HCV has been developed by the Chiron Corporation of Emeryville California. Before the availability of reliable serologic tests for hepatits C, a diagnosis of non-A, non-B hepatitis was made by serologic exclusion of HAV and HBV infection in the setting of a compatible history. Now that a specific antibody test is available, the potential exists for making a specific serologic diagnosis; however, delay;s of 1 to 3 months before the appearance of detectable antibody may interfere with serodiagnosis during acute illness. Furthermore, the level of antibody appears to be quite low. A helpful clinical clue is the episodic pattern of animotransferase elevation seen frequently in non-A, non-B hepatitis can be entertained if tests for HBsAg, IgM anti-HBc, and IgM anti-HAV are negative. If follow-up samples are obtained 1 or more months after the onset of acute illness, a specific serologic diagnosis of bloodborne non-A, non-B hepatitis (hepatitis C)may be made.

HDVis a defective RNA virus which coinfects with and requires the helper function of HBV for its replication and expression. Slightly smaller than HBV, HDV is a formalin-sensitive 35- to 37-nm virus with a hybrid structure. Its nucleocapsid expresses HDV antigen, which bears no antigenic homology with any of the HBV antigens, and contains a small, 1700-nucleotide RNA genome that is nonhomologous with HBV DNA but that has features of plant satellite viruses or viroids. This HDV core is "encapsidated" by an outer coat of of HBsAg. Thus, HDV can either infect a person simultaneously with HBV (coinfection) or superinfect a person already infected with HBV (superinfection); when HDV infection is transmitted from a donor with one HBsAg subtype to an HBsAg-positive recipient with a different subtype, the HDV agent assumes the HBsAg subtype of the recipient, rather than the donor. Because HDV relies absolutely on HBV, the duration of HDV infection is determined by the duration of and cannot outlast HBV infection. HDV antigen is expressed primarily in hepatocyte nuclei and is occasionally detectable in serum. During acute HDV infection, anti-HDV of the IgM class predominates; in self-limited infection, anti-HDV is low-titer and

transient, rarely remaining detectable beyond the clearance of HBsAg and HDV antigen. In chronic HDV infection, anti-HDV can be detected. HDV antigen in the liver and HDV RNA in serum and liver can be detected duringt HDV replication.

The presence of HDV infection can be identified by demonstrating intrahepatic HDV antigen or, more practically, an antiHDV seroconversion (a rise in titer of anti-HD or *de novo* appearance of IgM anti-HD). Circulating HDAg, also diagnostic of acute infection, is detectable only briefly, if at all. Because IgM is anti-HD is transient and IgG anti-HD is often undetectable once HBsAg disappears, retrospective serodiagnosis of acute self-limited, simultaneous HBv and HDV infection is difficult.

When a patient presents with acute hepatitis and has HBsAg and anti-HD in the serum, determination of the class of anti-HBc is helpful in establishing the relationship between infection with HBV and HDV. Although IgM anati-HBc does not distinguish absolutely between acute and chronic HBV infection, its presence is a reliable indicator of recent infection and its absence a reliable indicator of infection in the remote past. In simultaneious acute HBV and HDV infections, IgM anti-HBc will be detectable, while in acute HDV infection superimposed upon chronic HBV infection, anti-HBc will be of the IgG class.

Chronic persistent and chronic lobular hepatitis result from infections with HBV and HCV. In general, these are both nonprogressive disorders; hepatic failure is not seen and evolution into cirrhosis is exceedingly rare. Occasionally, however, patients with chronic active hepatitis may be seen during spontaneous remission, at which time the histopathologic findings may suggest chronic persistent or chronic lobular hepatitis. Under these circumstances relapses and progression to the more serious underlying chronic active hepatitis may occur. Another exception to the nonprogression of chronic persistent and lobular hepatitis occurs in patients positive to HBV surface antigen (HBsAg), in whom superinfection with HDV agentmay lead to the development of chronic active hepatitis. Indeed, the clinical

presentation of rapidly progressive liver injury in a known chronic HBsAg carrier should suggest infection with HDV.

It is an object of the present invention to produce a method for treating hepatitis, in particular chronic hepatitis B.

SUMMARY OF THE INVENTION

The present invention provides a method for treating hepatitis in a mammal in need of such treating comprising:

administering to such a mammal a therapeutically effective amount of granulocyte/macrophage-colony stimulating factor (GM-CSF) or a polypeptide analog thereof having substantially the same amino acid sequence and the activity of naturally occurring GM-CSF.

Preferably, the mammals treated will be humans and the GM-CSF utilized will be one of the human allotypes. Preferably, the GM-CSF will be administered in an amount of about 0.1 to 10 micrograms (µgs) per kilogram of body weight per day.

Another embodiment of the present invention is a method for treating hepatitis in a mammal in need of such treating in which GM-CSF is administered in conjunction with α -interferon.

BRIEF DESCRIPTION OF THE DRAWINGS

Fig. 1 shows ALT and HBV-DNA levels obtained from human patients who were afflicted with chronic hepatitis B and who were treated with 0.5 µgs of r GM-CSF per kilogram (Kg) of weight subcutaneously daily for 6 weeks. After two weeks rest period, each patient was then administered 3 million IU of recombinant α-Interferon (rIFN-α) subcutaneously daily for 6 additional weeks.

Fig. 2 shows ALT and HBV-DNA levels obtained from human patients who were afflicted with chronic hepatitis B and who were treated with 1.0

µgs of rGM-CSF per Kg of weight subcutaneously daily for 6 weeks. After two weeks rest period, each patient was then administered 3 million IU of rIFN-α subcutaneously daily for 6 additional weeks.

Fig. 3 shows ALT and HBV-DNA levels obtained from human patients who were afflicted with chronic hepatitis B and who were treated with 1.5 µgs of rGM-CSF per Kg of weight subcutaneously daily for 6 weeks. After two weeks rest period, each patient was then administered 3 million IU of rIFN-α subcutaneously daily.for 6 additional weeks.

DETAILED DESCRIPTION OF THE INVENTION

According to thepresent invention, a mammal, especially a human, can be effectively treated for hepatitis by the administration of a therapeutically effective amount of GM-CSF.

GM-CSF having substantially the same amino acid sequence and the activity of naturally occurring GM-CSF may be employed in the present invention. Complementary DNAs (cDNAs) for GM-CSF have been cloned and sequenced by a number of laboratories, e.g. Gough *et al.*, *Nature*, **309**: 763 (1984) (mouse); Lee *et al.*, *Proc. Natl. Acad. Sci. USA*, **82**: 4360 (1985) (human); Wong *et al.*, *Science*, **228**: 810 (1985) (human and gibbon); Cantrell *et al.*, *Proc. Natl. Acad. Sci. USA*, **82** : 6250 (1985) (human), Gough *et al.*, *Nature*, 309: 763 (1984) (mouse); Wong *et al.*, *Science*, 228:810 (1985) (human and gibbon); Cantrell *et al.*, *Proc. Natl. Acad. Sci. U.S.A.*, 82: 6250 (1985) (human). Among the human GM-CSFs, nucleotide sequence and amino acid heterogeneity have been observed. For example, both threonine and isoleucine have been observed at position 100 of human GM-CSF with respect to the N-terminal position of the amino acid sequence. These leader sequences may be of various lengths and amino acid compositions which may or may not affect biological activity. Preferably, the GM-CSF used in the present invention for treating humans will be human GM-CSF (hGM-CSF), most preferably the GM-CSF described in Lee *et al.*, *Proc. Natl.*

Acad. Sci. USA, 82: 4360 (1985), as purified in U.S. Patent Application No. 07/111,886 filed October 23, 1987.

GM-CSF can also be obtained from Immunex, Inc. of Seattle, Washington and Schering-Plough Corporation of Kenilworth, New Jersey.

Administration of GM-CSF can be subcutaneous, intravenous, nasal, parenteral, intramuscular, or any other acceptable method. The preferred mode of administration is subcutaneous. In general, a therapeutically effective amount of GM-CSF is about 0.1 to 10 micrograms (μg), preferably 0.5 to 3 μg of GM-CSF per kilogram of body weight of the patient per day. INF-α can also be administered to the patient in conjunction with GM-CSF. The term "in conjunction with" as used herein refers to the administration of INF-α co-temporaneously, during or immediately before or following administration of GM-CSF. If α-interferon (INF-α) is also administered, the therapeutically effective amount of the INF-α will be about 3 million international units (IU) which can also be administered subcutaneously per kilogram weight of the patient per day.

Human alpha interferon (h INF-α) is a naturally occurring mixture of at least eleven compounds including those designated alpha-1 and alpha-2 interferon.

A number of INF-α species or components are known and are usually designated by a numeral and letter after the Greek letter alpha. Human alpha-1 interferon is one species comtemplated for use in this invention as are the species designated human alpha-2 interferons. Under USAN, recombinant DNA human alpha-2 interferons are designated Interferon Alpha-2a, which can be made as disclosed in Rubenstein, Biochem. Biophys. Acta (1982), 695, 5-16, and Interferon Alpha-2b. Interferon Alpha-2b is the preferred species for use in this invention and is a recombinant DNA human alpha interferon (hereinafter rh INF-α). Another suitable rhIFN-α included in the scope of the present invention is recombinant DNA human interferon alpha-2a.

Human interferon alpha-2b can be produced in bacteria and other microorganisms using recombinant DNA techniques including those disclosed in Nagata *et al.* Nature, (1980) 284, 316-319; European Patent 32,134 and U.S. Patent No. 4,289,690. Various alpha interferon species are disclosed in U.S. Patent 4,503,035.

International Units are determined by comparison of the antiviral activity of the α-interferon which is used with the activity of the international reference preparation of human leukocyte interferon (α-interferon) established by the World Health Organization.

The formulations and pharmaceutical compositions contemplated by the above dosage forms can be prepared with conventional pharmaceutically acceptable excipients and additives, using conventional techniques.

Solutions of GM-CSF to be administered may be reconstituted from lypholized powders and they may additionally contain preservatives buffers, dispersants, etc. Preferably, GM-CSF is reconstituted with any isotonic medium normally utilized for subcutaneous injection, e.g., preservative-free sterile water. The maximum concentration of GM-CSF preferably should not to exceed 1500 micrograms per milliliter. Patients responding to treatment with GM-CSF as indicated by a drop in ALT levels should continue treatment from 16 to 24 weeks. The precise amount of the GM-CSF to be administered and the length of treatment would be determined by the attending clinicians, taking into account the etiology and severity of the disease, the patient's condition, sex, age, and other factors.

The effect of GM-CSF on the treatment of hepatitis is illutrated by the following non-limiting human clinical data which should not be construed to limit the scope of the disclosure.

Example

Treatment of Patients Afflicted with Chronic Hepatitis B with GM-CSF

Nine patients afflicted with chronic active hepatitis were divided into 3 groups each having 3 patients per group. The patients were both

male and female and their ages ranged from 18 to 60 years. The inclusion criteria for a patient to have been accepted into the present study were as listed below

1. The patients who were entered into the study must have had as a previous condition a liver biopsy performed within 6 months prior to the beginning of the study which resulted in histopathologically proven chronic liver disease with or without cirrhosis.

2. Evidence of HBV replication as shown by serum HBeAg and HBV-DNA positive data obtained at least during the six months before entry in the study.

3. Each patient had evidence of ongoing liver cell injury (AST/ALT elevation) for more than six months.

The recombinant GM-CSF which was used for the present example was obtained from the Schering-Plough Corporation of Kenilworth, New Jersey. The α-interferon which was used was interferon alfa-2b, INTRON A®, obtained from Schering-Plough of Kenilworth, New Jersey. The specific activity of INTRON A® is approximately 2×10^8 IU/mg protein.

Group 1

The patients in Group 1 each received 0.5 µgs of r GM-CSF per kilogram (Kg) of weight subcutaneously daily for 6 weeks. After two weeks rest period, each patient was then administered 3 million IU of rIFN-α subcutaneously daily for 6 additional weeks.

Results

The data as indicated in Figure 1 show that GM-CSF is marginally effective in treating chronic hepatitis at the dosage of 0.5µg/Kg weight per day as only one patient of the three showed any improvement at this dosage as indicated by either a decrease in the HBV-DNA level and/or by a decrease in the ALT level.

Group 2

The patients in Group 2 each received 1.0 µg of rGM-CSF per kilogram (Kg) of weight subcutaneously daily for 6 weeks. After two weeks rest period, each patient was then administered 3 million IU of rIFN-α subcutaneously daily for 6 additional weeks.

Results

The data as indicated in Figure 2 show that GM-CSF is effective in treating chronic hepatitis at the dosage of 1.0µg/Kg weight per day as all three patients showed some improvement as indicated by as indicated by either a decrease in the HBV-DNA level and/or by a decrease in the ALT level.

Group 3

The patients in Group 2 each received 1.5 µgs of r GM-CSF per kilogram (Kg) of weight subcutaneously daily for 6 weeks. After two weeks rest period, each patient was then administered 3 million IU of recombinant rIFN-α subcutaneously daily for 6 additional weeks.

Results

The data as indicated in Figure 3 show that GM-CSF is effective in treating chronic hepatitis at the dosage of 1.5µg/Kg weight per day as

2 of the three patients showed some improvement as indicated by either a decrease in the HBV-DNA level and/or by a decrease in the ALT level.

What is claimed is:

1. A method for treating hepatitis in a mammal comprising:
administering to the mammal in need of such treating a therapeutically effective amount of granulocyte/macrophage-colony stimulating factor (GM-CSF) or a polypeptide analog thereof having substantially the same amino acid sequence and the activity of naturally occurring GM-CSF sufficient for such treating.

2. The method of claim 1 wherein hepatitis B is treated.

3. The method of claim 1 wherein chronic active hepatitis B is treated

4. The method of claim 1 wherein the amount of GM-CSF which is administered to the mammal is from about 0.5 µgs to 10 µgs per kilogram weight of the mammal per day

5. The method of claim 1 wherein the GM-CSF is glycosylated.

6. The method of claim 1 wherein the GM-CSF is non-glycosylated.

7. The method of claim 1 further comprising administering α-interferon (INF-α) in conjunction with GM-CSF.

8. The method of claim 1 wherein the mammal is a human.

9. A method of treating hepatitis in a mammal in need of such treating comprising:
administering to such a mammal in need of such treatment with a therapeutically effective amount of granulocyte /macrophage-colony stimulating factor (GM-CSF) in conjunction with α-interferon (INF-α).

10. The method of claim 9 wherein the INF-α is administered before during or after the GM-CSF is administered.

11. The method of claim 9 wherein the amount of GM-CSF which is administered to the mammal is from about 0.5 µgs to 3 µgs per kilogram weight of the mammal per day and the amount of INF-α which is administered is about 3 million IU per day.

12. The use of GM-CSF in the manufacture of a pharmaceutical composition for treating hepatitis.

13. The use of GM-CSF to treat hepatitis.

14. A pharmaceutical composition for the treatment of hepatitis comprising GM-CSF.

FIG. 1A
PATIENT 1

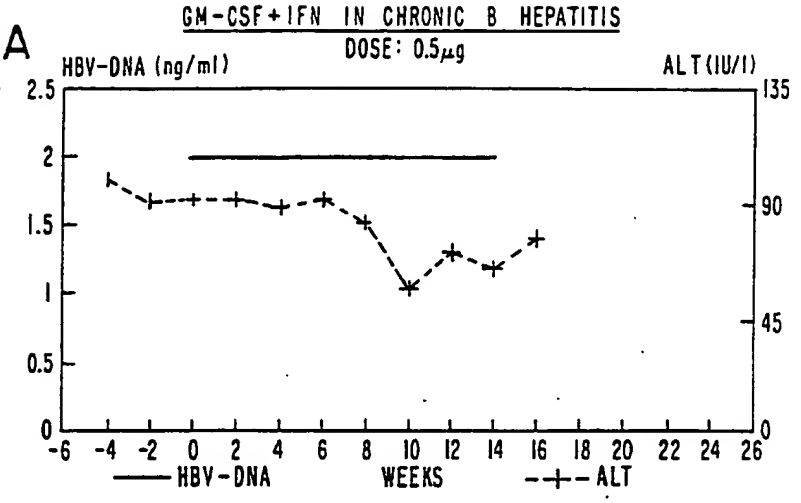


FIG. 1B
PATIENT 2

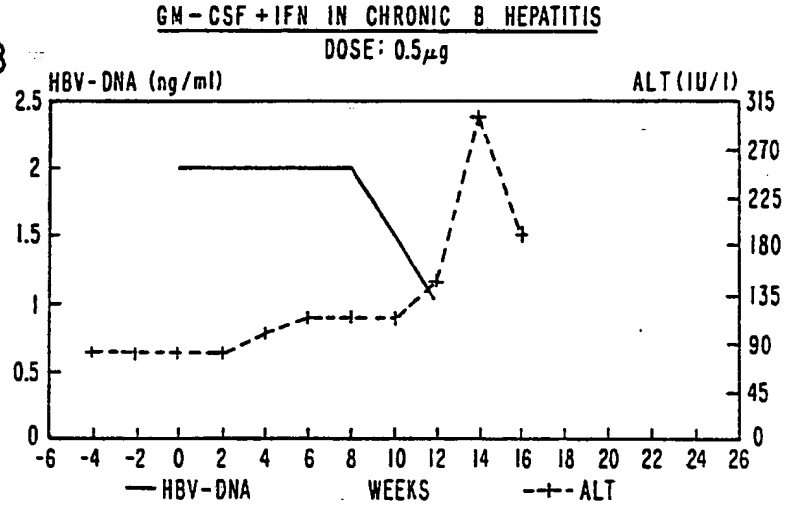


FIG. 1C
PATIENT 3

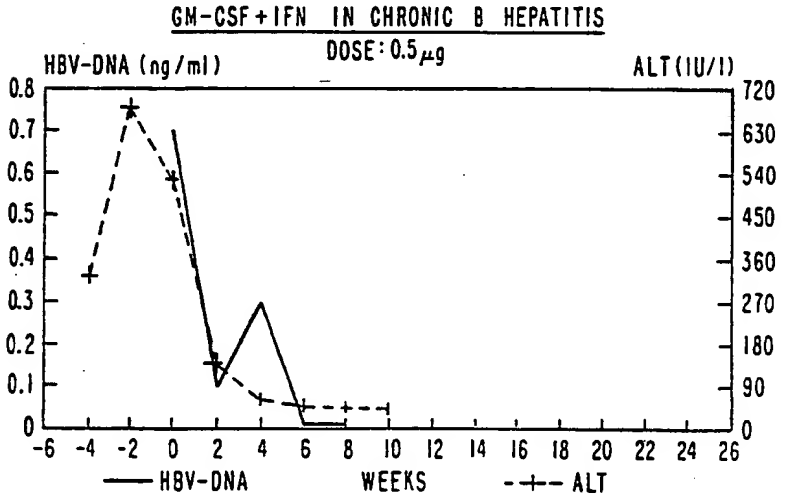


FIG. 2A

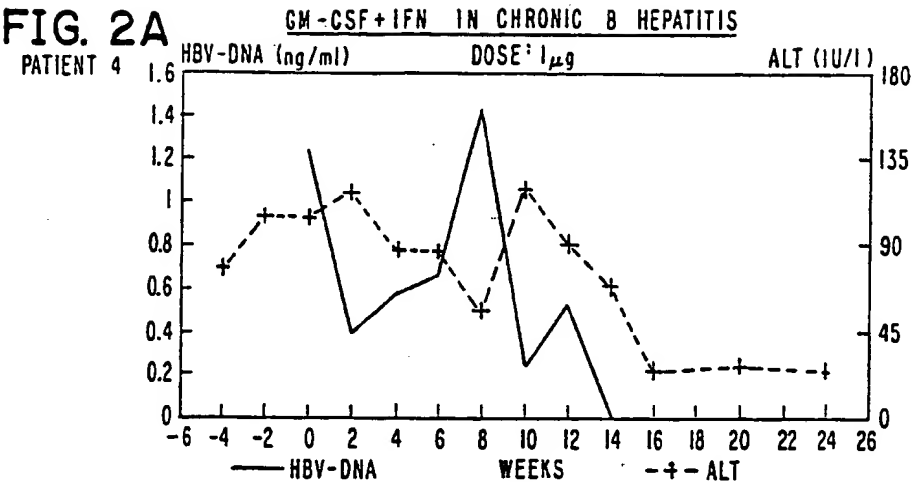


FIG. 2B

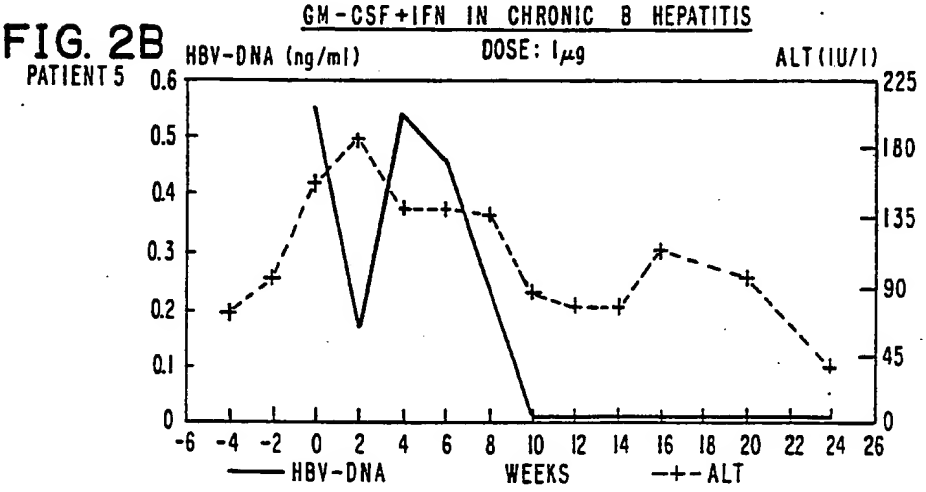


FIG. 2C

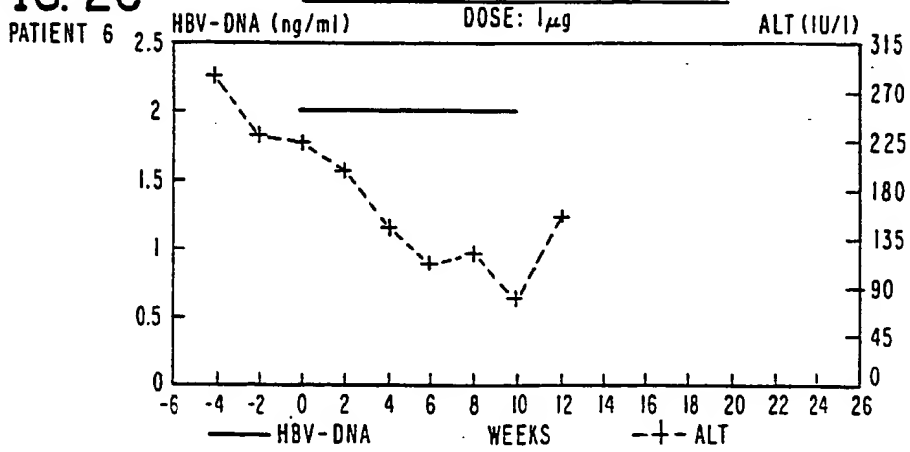


FIG. 3A
PATIENT 7

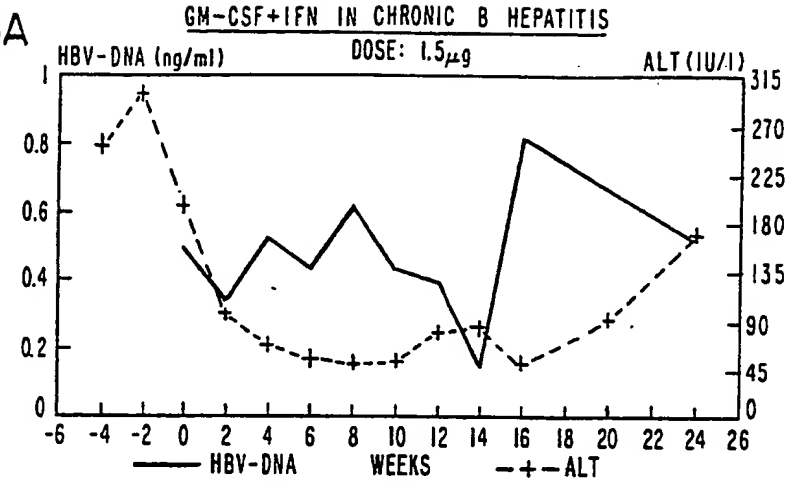


FIG. 3B
PATIENT 8

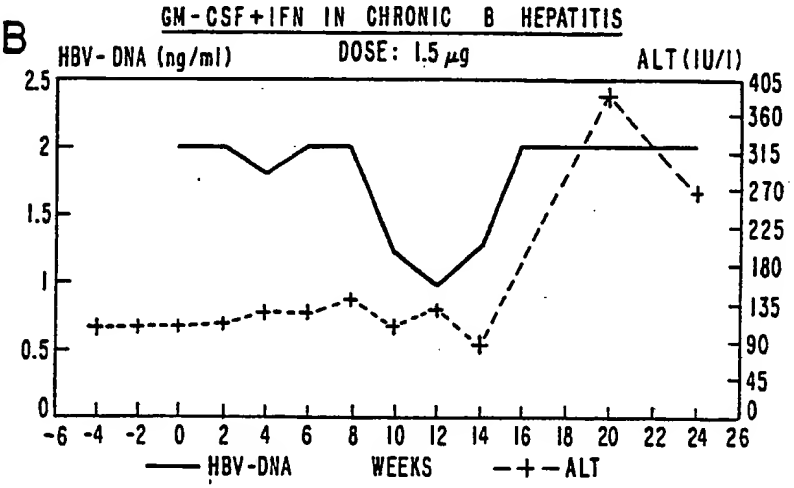
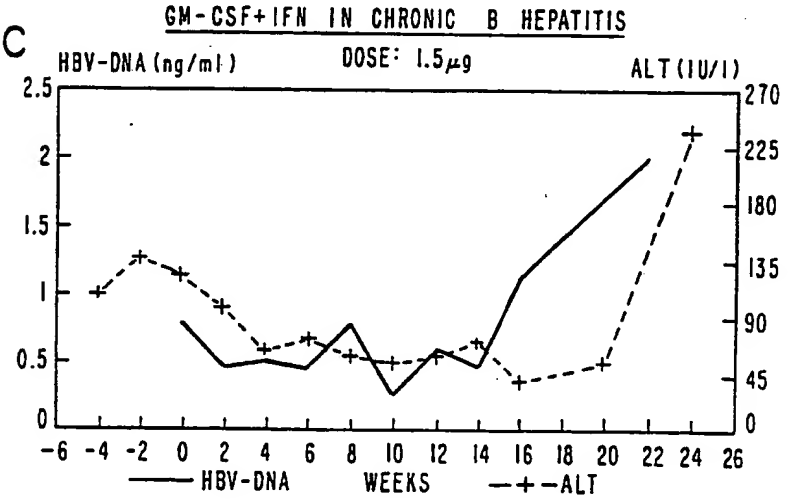


FIG. 3C
PATIENT 9



INTERNATIONAL SEARCH REPORT

International Application No PCT/US 93/03148

I. CLASSIFICATION OF SUBJECT MATTER (If several classification symbols apply, indicate all)[6]

According to International Patent Classification (IPC) or to both National Classification and IPC

Int.Cl. 5 A61K37/02; A61K37/66; //(A61K37/66,A61K37:02)

II. FIELDS SEARCHED

Minimum Documentation Searched[7]

Classification System	Classification Symbols
Int.Cl. 5	A61K ; C07K ; C12N ; C12P

Documentation Searched other than Minimum Documentation to the Extent that such Documents are Included in the Fields Searched[8]

III. DOCUMENTS CONSIDERED TO BE RELEVANT[9]

Category °	Citation of Document, [11] with indication, where appropriate, of the relevant passages [12]	Relevant to Claim No.[13]
A	HEPATOLOGY vol. 14, no. 4/2, 1991, page 216A JA. QUIROGA ET AL 'Immunological studies in patients with chronic hepatitis C. Factors affecting response to Interferon alpha' & 42ND ANNUAL MEETING OF THE AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES CHICAGO; USA. 2-5 NOVEMBER 1991. * see abstract no 674 * ---	1,7-11
A	EP,A,0 294 160 (SCHERING CORPORATION) 7 December 1988 see the whole document ---	1,7-11

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° Special categories of cited documents : [10]

"A" document defining the general state of the art which is not considered to be of particular relevance

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"T" later document published after the international filing date or priority date and not in conflict with the application but cited to understand the principle or theory underlying the invention

"X" document of particular relevance; the claimed invention cannot be considered novel or cannot be considered to involve an inventive step

"Y" document of particular relevance; the claimed invention cannot be considered to involve an inventive step when the document is combined with one or more other such documents, such combination being obvious to a person skilled in the art.

"&" document member of the same patent family

IV. CERTIFICATION

Date of the Actual Completion of the International Search	Date of Mailing of this International Search Report
01 JULY 1993	13. 07. 93
International Searching Authority	**Signature of Authorized Officer**
EUROPEAN PATENT OFFICE	LE CORNEC N.D.R.

III. DOCUMENTS CONSIDERED TO BE RELEVANT (CONTINUED FROM THE SECOND SHEET)

Category °	Citation of Document, with indication, where appropriate, of the relevant passages	Relevant to Claim No.
P,X	HEPATOLOGY vol. 16, no. 4/2, 1992, page 67 A J. MARTIN ET AL 'Recombinant human granulocyte macrophage colony stimulating factor (rhGM-CSF) in the treatment of chronic hepatitis B' & 43RD ANNUAL MEETING AND POSTGRADUATE COURSE OF THE AMERICAN ASSOCIATION FOR THE STUDY OF LIVER DISEASES. OCTOBER 31ST - NOVEMBER 3RD 1992. CHICAGO; USA. * see abstract 89 *	1-14
A	WO,A,9 004 973 (SCHERING CORPORATION) 17 May 1990 see abstract	1,14

INTERNATIONAL SEARCH REPORT

rnational application No.
PCT/US 93/03148

Box I Observations where certain claims were found unsearchable (Continuation of item 1 of first sheet)

This international search report has not been established in respect of certain claims under Article 17(2)(a) for the following reasons:

1. [X] Claims Nos.:
because they relate to subject matter not required to be searched by this Authority, namely:

Remark: Although claims 1-11,13 are directed to a method of treatment of the human/animal body the search has been carried out and based on the alleged effects of the compound/composition

2. [ ] Claims Nos.:
because they relate to parts of the international application that do not comply with the prescribed requirements to such an extent that no meaningful international search can be carried out, specifically:

3. [ ] Claims Nos.:
because they are dependent claims and are not drafted in accordance with the second and third sentences of Rule 6.4(a).

Box II Observations where unity of invention is lacking (Continuation of item 2 of first sheet)

This International Searching Authority found multiple inventions in this international application, as follows:

1. [ ] As all required additional search fees were timely paid by the applicant, this international search report covers all searchable claims.

2. [ ] As all searchable claims could be searches without effort justifying an additional fee, this Authority did not invite payment of any additional fee.

3. [ ] As only some of the required additional search fees were timely paid by the applicant, this international search report covers only those claims for which fees were paid, specifically claims Nos.:

4. [ ] No required additional search fees were timely paid by the applicant. Consequently, this international search report is restricted to the invention first mentioned in the claims; it is covered by claims Nos.:

Remark on Protest

[ ] The additional search fees were accompanied by the applicant's protest.

[ ] No protest accompanied the payment of additional search fees.

ANNEX TO THE INTERNATIONAL SEARCH REPORT ON INTERNATIONAL PATENT APPLICATION NO.

US 9303148
SA 72290

This annex lists the patent family members relating to the patent documents cited in the above-mentioned international search report.
The members are as contained in the European Patent Office EDP file on
The European Patent Office is in no way liable for these particulars which are merely given for the purpose of information. 01/07/93

Patent document cited in search report	Publication date	Patent family member(s)		Publication date
EP-A-0294160	07-12-88	WO-A-	8809673	15-12-88
WO-A-9004973	17-05-90	AU-B-	630637	05-11-92
		AU-A-	4526889	28-05-90
		CA-A-	2002413	09-05-90
		EP-A-	0370656	30-05-90
		EP-A-	0446230	18-09-91
		JP-T-	4500210	16-01-92